these stages will no doubt contribute to our understanding of the highly orchestrated events occurring during early embryogenesis.

Chromosome Organization in Meiosis

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Yeast chromosomes exhibit subdiffusive motion during meiosis.

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Our objective is to understand the mechanics of homologous chromosome pairing during meiosis. Aberrant pairing can result in nondisjunction and birth defects in humans.
This study used yeast, *Saccharomyces cerevisiae*, with chromosomally-integrated arrays of tetO operators that bind TetR repressor proteins fused to GFP to produce a fluorescent signal. In diploid cells, the tetO/TetR-GFP system allows homologous chromosomes to be identified as two foci (unpaired) or one focus (paired) as they progress through meiosis. We conducted three replicate timecourse experiments, analysing three different stages of meiosis, t=0 hours: pre-meiotic, t=3 hours: pairing transition, and t=5 hours: synapsis. At each stage, the cells were imaged for 25 minutes, with z-stacks taken at 30 second intervals. To analyse individual cells, we developed a 4D image analysis pipeline in MATLAB that allowed us to calculate the mean squared change in distance (MSCD), a metric describing the distance between two foci, and analyse deviations from normal diffusive motion.
At the t0 time point, chromosomes in 5% of cells were unpaired, 23% were paired, and 72% were "kissing", where they spent 885 ± 33 s as a single focus. At t3, there was an increase to 10% unpaired and 27% paired cells and the reduced 63% of kissing cells spent less time, 739 ± 110 s, in the paired state. At t5, the cells returned to a state similar to that observed prior to meiosis; 3% of cells were unpaired, 24% were paired and the remaining 72% of kissing cells spent 1007 ± 78 s paired. These results suggest that chromosomes are dynamic both prior to and during meiosis, with the greatest pairing instability occurring at t3.
When plotting MSCD over various time intervals, the curve plateaued at larger time scales. At t0, the maximum MSCD was 0.21 ± 0.02 μm^2, this increased to 0.38 ± 0.03 μm^2 at t3, and returned to 0.27 ± 0.04 μm^2 at t5. We fitted equations to the MSCD data and found that subdiffusion had lower residuals, 7E-4 ± 2E-4, than confined, 5E-3 ± 1E-3, or normal, 8E-2 ± 2E-2, diffusion. The α coefficient indicates how anomalous the motion is with $\alpha = 1$ being normal diffusion and $\alpha < 1$ being subdiffusive motion. We observed a highly subdiffusive α coefficient of 0.26 ± 0.03 at t0; the α value increased slightly (decreased subdiffusion) at t3 to 0.34 ± 0.03, and then returned to 0.27 ± 0.04 at t5.
Our image analysis pipeline has provided us with novel insights into the mechanics of homologous chromosome pairing: 1) homolog pairing and unpairing is dynamic even at late stages of meiosis; and 2) subdiffusive motion suggests a crowded nuclear environment or one with viscoelastic properties.